REMARKS

This amendment is responsive to the Office Action dated April 30, 2004. Claims 1-3, 5-7 and 12-13 are amended herein. Claim 8 has been canceled. New dependent claims 38-42 have been added. Claims 1-7, 9-20 and 38-42 will be pending in the present application upon entry of this amendment.

Claims 2 and 13 have been amended to correct typographical errors.

Claim 1 has been amended to specifically recite the claimed compounds that inhibit cell differentiation. Basis for this amendment can be found at page 4, line 14 through page 6, line 15. Claim 1 has also been amended to specifically recite the claimed antioxidants. Basis for this amendment is found at pages 7, line 4 through page 8, line 10 of the specification as originally filed.

Claim 6 has been amended to expressly recite the antioxidant enzymes, superoxide dismutase catalase, glutathione peroxidase and methionine reductase. Basis for this amendment is found on page 8, lines 4-6 of the specification as originally filed.

Claim 7 has been amended to replace references to flavonoids and flavonoid derivatives with a list of compounds found on page 13, lines 13-33 and page 14 lines 1-3 and in claim 8 of the specification as originally filed.

Claim 12 has been amended to remove the recitation of "anti-inflammatories".

New claims 38-41 are based on original claim 5. New claim 42 is based on page 3, lines 13-15 of the application as originally filed.

Information Disclosure Statements

The applicant noted that the Examiner has not returned a copy of the initialed form PTO-1449 from the Information Disclosure Statement that was filed on April 9, 2004. In addition, the applicant has filed supplemental Information Disclosure Statements on June 16, 2004 and June 24, 2004, in the above-identified application. The Examiner is requested to return the initialed forms PTO-1449 for these Information Disclosure Statements to the applicant along with the next communication in this application.

Rejections under 35 U.S.C. § 112

Claims 1, 7, and 12-19 have been rejected under 35 U.SC. § 112, first paragraph, for lack of enablement, because the specification, according to the Examiner,

> ...while being enabling for the particular compounds effective to regulate at least of one cell differentiation and cell proliferation such as vitamin D3, the particular antioxidants such as vitamin A, vitamin E or coenzyme Q10, the particular anti-inflammatories disclosed in the specification employed in the particular method of treatment of radiation injury, does not reasonably provide enablement for any substances or compounds represented by "one or more compounds effective to regulate at least one of cell differentiation and cell proliferation", "one or more antioxidants" and "anti-inflammatories" recited in the claims herein.

Office Action dated April 30, 2004, page 3. The Examiner goes on to state that the recitations of "one or more compounds effective to regulate at least one of cell differentiation and cell proliferation," "one or more antioxidants," "one or more antioxidant enzymes," and "anti-inflammatories" are seen to be merely functional language." Office Action dated April 30, 2004, page 4.

In response to this amendment, some of the objected to terminology has been deleted from the claims of the application, and, with respect to the remaining terms, Markush groups reciting specific antioxidants, and reciting specific compounds that inhibit one of cell proliferation and cell differentiation have been added to the claims. It is considered that these amendments obviate the rejections. Similar amendments were made in applicant's co-pending U.S. Patent application no. 10/132,642, to obviate similar rejections and these amendments were found by the same Examiner to obviate the rejections.

However, the applicant wishes to make it clear on the record that the applicant does not agree with the Examiner's position on the enablement issue for at least the reasons previously set forth during the prosecution of this application and thus does not concede that the Examiner's position is correct. Accordingly, withdrawal of the rejection of claims 1, 7, and 12-19 under 35 U.S.C. 112, first paragraph, on the basis of undue experimentation is respectfully requested.

The Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 2-3, 7, and 10 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite on the basis that these claims include the terminology, "analogs," "may be," "other," "derivatives" of various kinds of compounds are not clearly defined in the specification.

The reference to claim 10 in this rejection appears to be an error since claim 10 does not contain any of the objectionable terminology. Claim 10 recites only the element selenium, which clearly and distinctly claims the subject matter of this particular claim.

With respect to the rejections of claims 2-3 and 7, although applicant does not agree with the Examiner's position for at least the reasons previously set forth in this application, applicant has amended claims 2-3 to remove the objectionable terminology and has amended claim 7 to add a Markush group reciting specific flavonoids and flavonoid derivatives. Accordingly, withdrawal of the rejection of claims 2-3, 7, and 10 under 35 U.S.C. 112, second paragraph is respectfully requested.

Double Patenting Rejections

The Examiner has rejected claims 1-9 and 12-20 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-11 of U.S. Patent application no. 09/993,003, which issued June 22, 2004, as U.S. Patent No. 6,753,325. Applicant has filed herewith a Terminal Disclaimer in compliance with 37 C.F.R. § 1.321(c) over U.S. Patent no. 6,753,325 in order to obviate this rejection. Withdrawal of the obviousness-type double patenting is respectfully requested.

The Examiner has provisionally rejected claims 1, 4-9 and 12-20 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-40 of co-pending application 10/288,761. Because as the claims in the co-pending application have not yet issued and may be amended, Applicant hereby requests deferral of this rejection until such time as notice of allowance in said co-pending application is received. Applicant preserves its' right to traverse this rejection.

The Examiner has provisionally rejected claims 1, 4-9 and 12-20 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-40 of co-pending application 10/279,315. Because as the claims in the co-pending application have not yet issued and may be amended, Applicant hereby requests deferral of this rejection until

such time as notice of allowance in said co-pending application is received. Applicant preserves its' right to traverse this rejection.

Rejections under 35 U.S.C. § 103(a)

Claims 1 through 20 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over U.S. Patent No. 6,162,801, issued to Kita (hereinafter "Kita"), Bissett, D.L. *et al.*, *J. Soc. Cosmet. Chem.* **1992**, *43*, 85-92 (hereinafter "Bissett"), and Darr, D. *et al.*, *British Journal of Dermatology* **1992**, *127*, 247-253 (hereinafter "Darr"), in view of Shimoi, K., *et al.*, *Mutation Research* **1996**, *350*, 153-161 (hereinafter "Shimoi", of which a complete copy is submitted herewith) and U.S. Patent No. 5,776,460, issued to Kim et al. (hereinafter "Kim"). Applicant respectfully traverses this rejection for the reasons set forth below.

Applicant respectfully submits that the Official Action does not set forth a *prima facie* case of obviousness in support of the rejection under 35 U.S.C. § 103(a). According to M.P.E.P. § 2143,

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.
>
> The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. [*Citation omitted.*]

The Official Action cites only one reference, namely Kita, that sets forth the use of D vitamins to prevent or treat UV-induced damage to the eyes or skin. Kita, however, sets forth the **topical** use of D vitamins, and states that the D vitamins function as a UV-screen. That is, according to Kita, an external layer of vitamin D will protect the eyes or the skin against UV-induced damage at least in part by absorbing the UV radiation before it reaches the body. *See* Kita at col. 4, lines 40-44, and at col. 6, lines 17-20, *inter alia.*

By contrast, Applicant's claimed invention is a method of treating or reducing radiation injury by **orally** administering a composition comprising one or more compounds effective to

inhibit at least one of cell differentiation and cell proliferation, for example, a D vitamin, and one or more antioxidants. Kita does not teach or suggest that a D vitamin, administered **orally**, would provide any beneficial effect against UV-induced damage. Moreover, a skilled person reading Kita would not expect oral administration of a D vitamin to provide a beneficial effect against UV-damage because the invention of Kita relies on the D vitamin absorbing the UV radiation to prevent exposure of the skin to the harmful UV radiation. See col. 8, lines 49-54 of Kita. The Examiner has presented no evidence that orally administered vitamin D would be present on the surface of the skin so that it can absorb UV radiation in accordance with the teachings of Kita.

Moreover, no skilled person in the pharmaceutical arts would conclude that oral administration of a composition would be effective based on evidence that topical administration is effective for a similar purpose. It this were the case, people would be eating sunscreen to prevent sunburn and rubbing aspirin on their skin to treat headaches with the expectation that these treatments would work. Skilled persons, however, do not extrapolate oral utility from topical utility since it is well known that topical products act in a completely different manner than orally ingested products. Shimoi, cited by the Examiner, for example, was aware that flavonoids exhibited *in vitro* activity as antioxidants and yet, even then Shimoi considered it necessary to conduct experiments to determine whether the same flavonoids would exhibit antioxidant activity *in vivo*, thereby confirming that skilled persons do not even conclude from *in vitro* testing that the same activity will be present *in vivo.*

Finally, the skilled person would immediately and unequivocally conclude that oral administration of a D vitamin would likely be ineffective since the D vitamin would no longer be present on the skin and thus would not be interposed between the UV radiation and the skin as taught by Kita.

On page 14 of the Office Action, the Examiner points out that Kita, in summarizing the prior art, mentions that therapeutic vitamin D may be administered orally or by injection. See col. 1, lines 42-44 of Kita. However, the prior art oral administration mentioned by Kita and relied on by the Examiner is not for the purpose of treating radiation injury, but rather, is for the purpose of treating one or more conditions selected from, "...rickets, osteomalacia, osteoporosis, osteatis, fibrosa, osteosclerosis and other bone diseases, and malignant tumors such as breast and colon cancers..." See col. 1, lines 16-21 of Kita. Again, this provides the skilled

person with no teaching or suggestion that oral administration of vitamin D would have any beneficial effect in the treatment of radiation injury.

Another problem with the Examiner's position is the title of Kita, which reads, "**External** Opthalmic Preparation Containing Vitamin D." From this, it is clear that Kita does not teach or suggest the internal administration of Vitamin D, as in the present invention.

On page 15 of the Office Action, the Examiner also takes the position that because Kita teaches that, "In general, the ultraviolet (UV) light absorption spectra of vitamin D and active vitamin D have absorption maxima at 265 nm, with the molar absorption coefficients of about 18900," one of skill in the art would have found it obvious to administer a vitamin D orally in treating radiation injury in a human. The applicant disagrees since if the vitamin D is administered orally, it will be internal to the body. As a result, the vitamin D will not be located between the body or skin and the UV radiation and thus the vitamin D will not be in the path of the UV radiation and therefore would not be able to absorb it. Also, it does not appear possible for the orally administered vitamin D to absorb UV radiation since the Examiner has not made a showing that the orally administered vitamin D would be present on the skin in order to absorb UV radiation. Skilled persons are aware that the process of absorption requires contact between the absorbent and the UV radiation in order to occur. In the case of oral administration of vitamin D, there is no evidence in the record that there would be any contact between the absorbent, vitamin D, and the UV radiation, and thus the vitamin D would not be expected absorb the UV radiation if administered orally, as is required for it to perform its absorbent function according to Kita. See e.g. col. 8, lines 49-54 of Kita.

None of the other references cited by the Examiner teach or suggest that oral administration of vitamin D would provide any beneficial effect in the treatment of radiation injury. Therefore, none of the cited references provides a reasonable expectation of success for the claimed invention, in view of the arguments given above.

In the Office Action the Examiner also takes the position that,

> "Additionally, oral administrations of vitamin D are well-known in the art. Thus, oral administration of vitamin D would inherently treat radiation injury in a human under the doctrine of inherency."

See page 15 of the Office Action. Even if the Examiner's statement is true, MPEP §2141.02 points out that,

> Obviousness cannot be predicated on what is not known at the time an invention is made, even if the inherency of a certain feature is later established. *In re Rijckaert*, 9 F.2d 1531, 28 USPQ2d 1955 (Fed. Cir. 1993).

A similar situation also arose in the case of In re Marshall, 578 F.2d 301, 198 USPQ 344 (CCPA 1978) (copy enclosed). In this case, the prior art disclosed the use of oxethazaine to inhibit the release of the acid-stimulating hormone, gastrin, in order to treat esophagitis, gastritis, peptic ulcer and irritable colon syndrome. The Boards of Appeal had taken the position that this reference inherently anticipated the applicant's claims directed to a method for the use of oxethazaine to inhibit release of the pancreatic secretory hormones, secretin and pancreozymin, in order to control weight on the basis that a person taking oxethazaine to inhibit the release of gastrin would inherently inhibit release of secretin and pancreozymin.

The Court of Customs and Patent Appeals, in the decision of In re Marshall, cited infra, disagreed with this reasoning and reversed the rejection of the Board of Appeals stating that if anyone ever lost weight by following the teachings of the prior art reference to take oxethazaine to inhibit release of gastrin, it was an unrecognized accident. The court then said that, "An accidental or unwitting duplication of an invention cannot constitute an anticipation." The same reasoning applies to the Examiner's rejection in the present case.

More specifically, the Examiner has taken the position that since the prior art discloses methods of administration of vitamin D to humans, a person following the teachings of the prior art would inherently treat radiation injury when the vitamin D is administered to a human exposed to radiation. However, as in In re Marshall, cited infra, if anyone ever treated radiation injury by following the teachings of the prior art relied on by the Examiner, it was an unrecognized accident, since none of the prior art relied on by the Examiner teaches or suggest that orally administered vitamin D is to be used to treat radiation injury. Although In re Marshall was decided in the context of an anticipation rejection, In re Rijckaert makes it clear that the same reasoning applies to an obviousness rejection. Thus, under the reasoning of In re Marshall and In re Rijckaert, this rejection should be withdrawn for this additional reason.

Accordingly, since it was not known at the time the present invention was made that vitamin D, ingested orally, would provide any beneficial effect for the treatment of radiation injury, the obviousness rejection based on inherency must fail.

In this regard, the Examiner relies on Eli Lilly and Co. v. Barr Laboratories, Inc., 251 F.3d 955; 58 USPQ2d 1869-1881 (Fed. Cir. 2001). However, the facts of that case are different than the facts in the present case. Specifically, the court in Eli Lilly found that,

> "Serotonin uptake inhibition is a natural biological activity that occurs when fluoxetine hydrochloride is administered to an animal, such as a human, for any purpose, including the treatment of anxiety. That is, serotonin uptake inhibition is an inherent property of fluoxetine hydrochloride upon its administration. Barr has offered [**37] a panoply of evidence to support the recognition of this inherent biological function of fluoxetine hydrochloride." (emphasis added)

The key difference between Eli Lilly and the present case is that in the present case, vitamin D does not treat radiation injury when administered for any purpose since, radiation injury is not always present in the person to whom the vitamin D is administered. In fact, in Eli Lilly there was a panoply of evidence supporting that fluoxetine hydrochloride always inhibited serotonin uptake. In the present case, there is no evidence whatsoever of any instance where orally administered vitamin D treated radiation injury. Accordingly, the facts of Eli Lilly were totally different than in the present case and thus the rationale of Eli Lilly does not apply. Rather, the facts of the present case are most similar to of In re Marshall and In re Rijckaert, discussed above, and thus the rejection should be withdrawn for the reasons given above.

On pages 15-16 of the Office Action, the Examiner also took the position that,

> ...since all active composition components herein are known to be useful to treat radiation injury, it is considered *prima facie* obvious to combine them into a single compositions to form a third composition useful for the very same purpose. At least additive therapeutic effects would have been reasonably expected.

There are several problems with this reasoning. First, the claims of the present application are not directed to a composition. Thus, whether it would have been obvious to make a composition comprising vitamin D and an antioxidant is irrelevant to the patentability of the claims of the present application. The key question is whether it would have been obvious to orally administer the composition of the present invention with the expectation of achieving a beneficial effect against radiation injury. See e.g. MPEP §2143 quoted above. The answer to the key question is that it would not be obvious since none of the cited references provides an expectation that oral administration of vitamin D would provide a beneficial effect for treatment of radiation injury, nor do any of the references provide a motivation to combine an antioxidant and vitamin D for use in oral administration for the treatment of radiation injury.

The second problem with the Examiner's reasoning is that the Examiner has already taken the position that, "...the pharmaceutical art is unpredictable requiring each embodiment to be individually assessed for physiological activity." See page 5, lines 18-20 of the Office Action. This statement directly contradicts the Examiner's conclusion that, "At least additive therapeutic effects would have been reasonably expected," found at page 14, lines 11-13 of the Office Action. The reason for this is that the Examiner has admitted that each embodiment must be individually assessed for physiological activity. As a result, according to the Examiner's own reasoning, it would not be possible to expect a particular therapeutic effect due to the unpredictability of the pharmaceutical art.

The remaining references cited in the Official Action, that is, Bissett, Darr, Shimoi, and Kim, include descriptions of the use of various antioxidants, or an antioxidant in conjunction with an anti-inflammatory, to treat radiation-induced damage. None of the cited references, however, includes any teaching or suggestion to combine the antioxidants with a compound effective to inhibit at least one of cell differentiation and cell proliferation, nor do any of these references teach or suggest that such a combination should be orally administered for the treatment of radiation injury. Also, none of these references includes any teaching or suggestion regarding the D vitamins, or oral administration of the D vitamins.

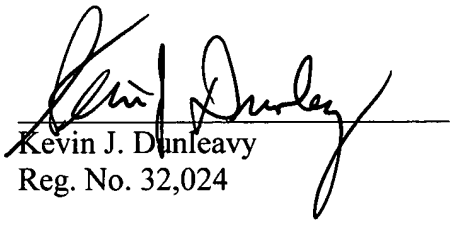
Applicant submits that the cited references do not contain every element of a *prima facie* case of obviousness, as discussed above. Accordingly, Applicant submits that the Official Action does not set forth a *prima facie* case for the obviousness of claim 1 over the cited references.

All of the dependent claims currently pending in the present application ultimately depend from independent claim 1. Applicant respectfully submits that, because independent claim 1 is not obvious over the cited references, dependent claims 2-7, 9-20 and 38-42 are also not obvious. Accordingly, Applicant respectfully requests that the rejection under 35 U.S.C. § 103(a) be withdrawn upon reconsideration.

Finally, new claim 42 specifically recites the types of radiation responsible for the claimed radiation injury. Claim 42 excludes UV radiation from the types of radiation that may be responsible for the claimed radiation injury. Thus, new claim 42 is considered to be separately patentable for this additional reason since the disclosure of Kita relative to the use of vitamin D is limited to providing protection from UV radiation.

In view of the foregoing amendments and remarks, Applicant respectfully submits that all of the pending claims are in condition for allowance and respectfully requests a favorable Office Action so indicating.

Respectfully submitted,



Dated: July 30, 2004

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IN THE MATTER OF THE APPLICATION OF EDWARD M. MARSHALL

Appeal No. 77-625.

UNITED STATES COURT OF CUSTOMS AND PATENT APPEALS

578 F.2d 301; 1978 CCPA LEXIS 270; 198 U.S.P.Q. (BNA) 344

June 30, 1978, Decided

PRIOR HISTORY: [**1]

Serial No. 468,552.

CASE SUMMARY:

PROCEDURAL POSTURE: Patent applicant appealed from the decision of the Patent and Trademark Office Board of Appeals which rejected applicant's claims on grounds of anticipation, *35 U.S.C.S. § 102,* and obviousness, *35 U.S.C.S. § 103.*

OVERVIEW: The Patent and Trademark Office Board of Appeals sustained the examiner's rejection of patent applicant's claims for a patent describing use of certain drugs in a weight loss program. Applicant appealed and the court reversed, saying that the applicant's claims were neither anticipated or obvious under the prior art. The court said that rejection based on anticipation required all material elements of a claim to be disclosed in a single piece of prior art. It was improper to combine the teachings of multiple existing works to support a finding of anticipation. As to obviousness, the court said that although the function of the drugs described was known, it had never been described as being useful for weight loss and so was not obvious. Applicant's use actually took advantage of what was thought to be a disadvantage of the drug.

OUTCOME: The court reversed the rejection of applicant's patent claims on the grounds of anticipation because no single piece of prior art contained all the material elements of the claims, and on grounds of obviousness because the claims described a new and unanticipated use for an existing drug.

LexisNexis (TM) HEADNOTES - Core Concepts:

Patent Law > Novelty & Anticipation
[HN1] Rejections under *35 U.S.C.S. § 102* are proper only when the claimed subject matter is identically disclosed or described in the prior art. In other words, to constitute an anticipation, all material elements recited in a claim must be found in one unit of prior art.

Patent Law > Novelty & Anticipation
[HN2] An accidental or unwitting duplication of an invention cannot constitute an anticipation.

Patent Law > Nonobviousness > Tests & Proof of Obviousness
[HN3] Known disadvantages of a drug which would naturally discourage the search for new uses of that drug may be taken into account in determining obviousness.

COUNSEL:

Edward D. O'Brian, attorney of record, for appellant.

Joseph F. Nakamura for the Commissioner of Patents, *Kack E. Armore,* of counsel.

OPINIONBY:

LANE

OPINION: [*302]

Before MARKEY, Chief Judge, RICH, BALDWIN, LANE, and MILLER, Associate Judges.

LANE, Judge.

This is an appeal from the decision of the Patent and Trademark Office (PTO) Board of Appeals (board) sustaining the examiner's rejection under *35 USC 102* of claims 1-4 and entering a new ground of rejection under *37 CFR 1.196(b)* of claims 5-9 under *35 USC 103*. We reverse both rejections.

BACKGROUND

Invention

Normally, when food passes through the terminal region of the stomach, nerve endings there stimulate the release of two hormones, secretin and pancreozymin. These hormones then trigger the production and release of pancreatic enzymes necessary for digestion in the small intestine.

Applicant's weight control process involves anesthetizing these nerve endings with an orally administered anesthetic containing 50-2,000 mg of oxethazaine. This prevents the release of secretin and pancreozymin which in turn interferes with the production and release of the pancreatic enzymes. [**2] Thus, food passing through the small intestine is not digested and does not contribute calories to the body.

The following claims are before us on appeal:

1. In a weight control process in which a quantity of food is consumed and passes through the gastro intestinal digestive tract of a living body the improvement which comprises:

said quantity of food including foodstuffs requiring digestion caused by pancreatic enzymes for absorption into the bloodstream from the small intestine,

periodically anesthesizing [sic] the nerve endings in the digestive tract which release hormones when contacted by food passing through the digestive tract so as to trigger the release of said pancreatic enzymes into the digestive tract by the pancreas prior to said quantity of food contacting said nerve endings only prior to the passage of food into said digestive tract, said anesthetization being carried out to an extent effective and at a time effective to inhibit said nerve endings from releasing sufficient hormones to cause the release of said pancreatic enzymes which will contact said food as it passes through the digestive tract,

said anesthetization serving to prevent the release of [**3] said hormones when said nerve endings are contacted by said quantity of food, this having the effect of preventing release of said enzymes by the pancreas to the digestive tract so that said food passes through the digestive tract without being digested so that it is [sic not] capable of being absorbed into the bloodstream as a consequence of the absence of said enzymes.

2. A weight control process as claimed in claim 1 wherein:

said nerve endings are anesthesized [sic] by orally taking a quantity effective to cause said inhibition of an anesthetic means coated with a coating means which is effective to delay the release of said anesthetic means until said anesthetic means reaches the vicinity of said nerve endings in the digestive tract.

3. A weight control process as claimed in claim 2 wherein:

said anesthetic means is oexthazaine.

4. A weight control process as claimed in claim 2 wherein:

said anesthetic means is orally taken with an adherence means for causing said anesthetic means to adhere to the interior of the digestive tract.

5. A weight control process as claimed in claim 4 wherein:

said adherence means is albumin and is admixed with said anesthetic [**4] means, said anesthetic means and said albumin both being coated with said coating means. [*303]

6. A weight control process as claimed in claim 2 wherein:

from about 50 to about 2,000 milligrams of said anesthetic means are taken at one time, said time being prior to food being taken into the digestive tract.

7. A weight control process as claimed in claim 2 wherein:

from about 200 to about 800 milligrams of said anesthetic means are taken at one time, said time being prior to food being taken into the digestive tract.

8. A weight control process as claimed in claim 1 wherein:

said nerve endings are anesthesized [sic] by orally taking a quantity effective to cause said inhibition of an anesthetic means coated with a coating which will delay

the release of said anesthetic means until said anesthetic means reaches the vicinity of said nerve endings in the digestive tract,

said anesthetic means is oxethazaine, and from about 50 to about 2,000 milligrams of said anesthetic means are taken at one time, said time being prior to food being taken into the digestive tract.

9. A weight control process as claimed in claim 8 wherein:

said anesthetic means is orally [**5] taken with adherence means for causing said anesthetic [sic means] to adhere to the interior of the digestive tract, and

said adherence means is albumin and is admixed with said anesthetic [sic means], said anesthetic [sic means] and said albumin both being coated with said coating.

Prior Art

The references relied upon are: the PHYSICIAN'S DESK REFERENCE 1522-23 (25th ed. 1971) (PDR); and J. Slayback, E. Swena, J. Thomas, L. Smith, The Pancreatic Secretory Response to Topical Anesthetic Block of the Small Bowel, 61 SURGERY 591 (1967) (Slayback).

The PDR describes drugs containing the anesthetic eo oxethazaine for the treatment of esophagitis, gastritis, peptic ulcer and irritable colon syndrome. The recommended adult oral dose of these drugs is one or two teaspoons (10-20 mg oxethazaine) four times daily, fifteen minutes before meals and at bedtime. The PDR expressly warns against exceeding the recommended dosage. Regarding the use of these drugs in the treatment of peptic ulcer, the PDR explains that topical application of this local anesthetic inhibits the release of the acid-stimulating hormone, gastrin.

Slayback is an article reporting an investigation into [**6] the mechanism responsible for the release of the pancreatic secretory hormones, secretin and pancreozymin. Researchers found that application of the anesthetic oxethazaine HCL to isolated segments of the small intestine of surgically altered dogs caused a substantial reduction in the release of both secreting and pancreozymin. These results were consistent with the hypothesis that secretin and pancreozymin release is controlled by a local neural mechanism similar to the one which had been shown to control the release of the gastric secretory hormone, gastrin.

Proceedings Below

The examiner rejected claims 1-4 under *35 USC 102* as anticipated by the PDR and also rejected claims 1-9 under *35 USC 102*/103 as anticipated or obvious over a patent to Pober. n1/ The board affirmed the 102 rejection of claims 1-4 but reversed the 102/103 rejection of claims 1-9 and entered a new ground of rejection under *37 CFR 1.196(b)* rejecting claims 5-9 under *35 USC 103* as obvious in view of the combined teachings of PDR and Slayback. n2/

n1/ U.S. patent No. *3,740,440*, issued June 19, 1973, for "Method of Inhibiting Appetite for Food."

n2/ The board does not explain why this new ground of rejection was not applied to claims 1-4 as well. [**7] [*304]

OPINION

102 Rejection

[HN1] Rejections under *35 USC 102* are proper only when the claimed subject matter is identically disclosed or described in the prior art. *In re Arkley, 59 CCPA 804, 807, 455 F.2d 586, 587, 172 USPQ 524, 526 (1972).* In other words, to constitute an anticipation, all material elements recited in a claim must be found in one unit of prior art. *Soundscriber Corp. v. United States, 360 F.2d 954, 960, 148 USPQ 298, 301 (Ct.Cl. 1966).* This basic principal of patent law has not been disturbed by our recent decision, *In re Samour, 571 F.2d 559, 197 USPQ 1 (CCPA 1978),* in which we affirmed a § 102(b) rejection of claims to a chemical compound based on a primary reference which disclosed the compound and additional references which established that a method of preparing the compound would have been obvious to one skilled in the art. In Samour, every material element of the claimed subject matter, the chemical compound, could be found in the primary reference, a disclosure of that compound.

Applying this rule of law to the present case, we must reverse the board's rejection of claims 1-4 under *35 USC 102* since the primary reference, the PDR, does not disclose [**8] every material element of the claimed subject matter. These claims are directed to a weight control process. Applicant uses an effective amount of the anesthetic, oxethazaine, to inhibit release of the pancreatic secretory hormones, secretin and pancreozymin, in order to control weight. The PDR, however, teaches using drugs containing the anesthetic oxethazaine to inhibit release of the acid-stimulating hormone, gastrin, in order to treat esophagitis, gastritis, peptic ulcer and irritable colon syndrome. Nothing in the PDR remotely suggests taking oxethazaine to lose weight. If anyone ever lost weight by following the PDR teachings it was an unrecognized accident. [HN2] An accidental or unwitting duplication of an invention

cannot constitute an anticipation. *In re Felton, 484 F.2d 495, 500, 179 USPQ 295, 298 (CCPA 1973).*

103 Rejection

The board seems to have combined: (1) the teaching of the PDR that oral administration of oxethazaine inhibits release of gastrin, (2) the teaching of Slayback that secretin and pancreozymin release is controlled by a local neural mechanism similar to the one which controls release of gastrin, and (3) the art-recognized fact that secretin and pancreozymin [**9] control the production and release of pancreatic enzymes necessary for digestion in the small intestine, to conclude that applicant's method of controlling weight by anesthetizing the nerve endings that stimulate the release of secretin and pancreozymin would have been obvious.

The problem with this rejection is that nowhere in any reference is there any suggestion to control weight by turning off the production and release of pancreatic enzymes. Although it has long been known that pancreatic enzymes are involved in digestion, from this record it appears that applicant is the first to suggest controlling weight by decreasing the quantity of pancreatic enzymes in the small intestine. To say this would have been obvious is to resort to impermissible hindsight.

Moreover, the PDR appears to teach away from using effective amounts of the anesthetic oxethazaine since it expressly cautions against exceeding the recommended does of 10-20 mg. This would not be an effective amount for controlling weight by appellant's process. Although Slayback, which discusses tests conducted solely on dogs, recognizes that higher concentrations of oxethazaine will produce "complete absence of stimulation [**10] of hormonal release," this does not negate the PDR warning with respect to the oral administration to humans. [HN3] Known disadvantages of a drug which would naturally discourage the search for new uses of that drug may be taken into account in determining obviousness. See *United States v. Adams, 383 U.S. 39, 52 (1966).* [*305]

Accordingly, for the reasons set forth herein, the decision of the board is reversed. n3/

n3/ The board rejected only claims 5-9 under *35 USC 103.* In the interest of judicial economy, we note that our reversal of that rejection is not based on any limitations of claims 5-9 not found in broader claims 1-4 as well.

REVERSED

DISSENTBY:

MARKEY (In Part)

DISSENT:

MARKEY, Chief Judge, dissenting-in-part, with whom BALDWIN, J., joins.

Though I wholeheartedly agree with the majority's treatment of the § 102 issue, I respectfully dissent from the majority's conclusion of non-obviousness under § 103.

The majority agrees that the board considered "the art recognized fact that secretin and pancreozymin control the production and release of pancreatic enzymes necessary for digestion in the small intestine." Nowhere in the record is there any dispute on that point. [**11] Moreover, the majority also recognizes that "it has long been known that pancreatic enzymes are involved in digestion."

Appellant and all others having ordinary skill in the art knew that pancreatic enzymes play a major role in the digestion of food. If food is not digested, it is excreted without being absorbed into the body. If food is not absorbed, the body cannot gain weight. It follows, therefore, that decreasing pancreatic enzyme quantity (or eliminating it altogether) must decrease weight. The particular compound chosen by appellant to shut off or decrease the flow of pancreatic enzymes was known in the art and used for that purpose.

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Fundamental and Molecular Mechanisms of Mutagenesis

Radioprotective effects of antioxidative plant flavonoids in mice

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Abstract

Radioprotective effects of tea infusions and plant flavonoids were investigated by using the micronucleus test for anticlastogenic activity and the thiobarbituric acid assay for antioxidative activity. A single gastric intubation of rooibos tea (*Aspalathus linearis*) infusion at 1 ml per mouse 2 h prior to γ-ray irradiation (1.5 Gy) reduced the frequency of micronucleated reticulocytes (MNRETs). After the fractionation of rooibos tea infusion, the flavonoid fraction was found to be most anticlastogenic and antioxidative. From this fraction, luteolin was isolated as an effective component. Then, anticlastogenic effects of 12 flavonoids containing luteolin and their antioxidative activities against lipid peroxidation by Fenton's reagent were examined. A good correlation ($r = 0.717$) was observed between both activities. Luteolin showed the most effective potency. A gastric intubation of luteolin (10 μmol/kg) 2 h prior to γ-ray irradiation (6 Gy) suppressed lipid peroxidation in mouse bone marrow and spleen and a trend of protective effect of luteolin against the decrease of endogenous ascorbic acid in mouse bone marrow after γ-ray irradiation (3 Gy) was observed. These results suggest that plant flavonoids, which show antioxidative potency in vitro, work as antioxidants in vivo and their radioprotective effects may be attributed to their scavenging potency towards free radicals such as hydroxyl radicals. Therefore, the flavonoids contained in tea, vegetables and fruits seem to be important as antioxidants in the human diet.

Keywords: γ-Irradiation; Radioprotection; Flavonoids; Anticlastogen; Antioxidant

1. Introduction

Epidemiological studies have shown that the consumption of vegetables, fruits and teas are associated with low risks of human cancer (Steinmetz and Potter, 1991; Block et al., 1992; Yang and Wang, 1993). Furthermore, it has been reported that diets lacking fruits and vegetables led to increases urinary biomarkers of oxidative DNA base damage (Simic and Bergtold, 1991). Oxidative damage of DNA, protein and lipids arises from the generation of free radicals by exogenous chemicals, radiation or endogenous oxidative stress. Oxygen radicals are involved in mutation, chromosome aberration, tumor promotion and cancer development. Antioxidative nutrients in vegetables and fruits such as ascorbic acid and β-carotene have traditionally been regarded as cancer preventive agents. They are thought to play an important role in protection against oxidative damages. Recently, however, the minor nonnutrients – organosulfur compounds, polyphenolic compounds and flavonoids – have been focused upon as anticarcinogens (Wattenberg, 1985). Flavonoids are widely

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distributed in the plant kingdom and are strong antioxidants (Letan, 1966; Nakatani, 1990). In addition, many studies of tea catechins have demonstrated strong antioxidative properties and inhibitory effects against tumor formation and growth (Yang and Wang, 1993).

In our previous paper, we demonstrated that rooibos tea (*Aspalathus linearis*) infusion reduced the frequency of micronucleated reticulocytes (MNRETs) in mice exposed to γ-rays (Shimoi et al., 1994a). It is known that γ-rays generate hydroxyl radicals in organisms and induce cellular DNA damage which leads to mutations and chromosomal aberrations (Kasai et al., 1986; Riley, 1994). In this study, radioprotective effects of tea infusions and plant flavonoids in mice were investigated. γ-Rays were used as the oxidative DNA damaging agent. We isolated effective, antimutagenic, flavonoids from rooibos tea using the micronucleus test for anticlastogenic activity and the thiobarbituric acid assay for antioxidative activity. We also examined whether the flavonoids work as antioxidants in vivo or not, because in vivo studies of the effect of flavonoids on antioxidative activity are few. Further, the relationship between the antioxidative activity and the anticlastogenic activity is discussed.

2. Identification of the radioprotective components from rooibos ten infusion

Rooibos tea is cultivated in the Cederberg area of the Cape Province of South Africa and is consumed as an herb beverage in South Africa and Europe. This tea contains no caffeine and little tannin but has many flavonoids (Blommaert and Steenkamp, 1978; Morton, 1983). The content of catechins is low in comparison with green tea (Kinae et al., 1994). This tea is believed to possess various physiological properties such as calming digestive disorders and reducing nervous tension. Recently, scavenging ability against active oxygen species (Yoshikawa et al., 1990) and inhibitory effects in X-ray induced cell transformation (Komatsu et al., 1994) were reported.

2.1. Fractionation of tea infusion

The procedure employed and the yields of each fraction are illustrated in Fig. 1. Rooibos tea infusion

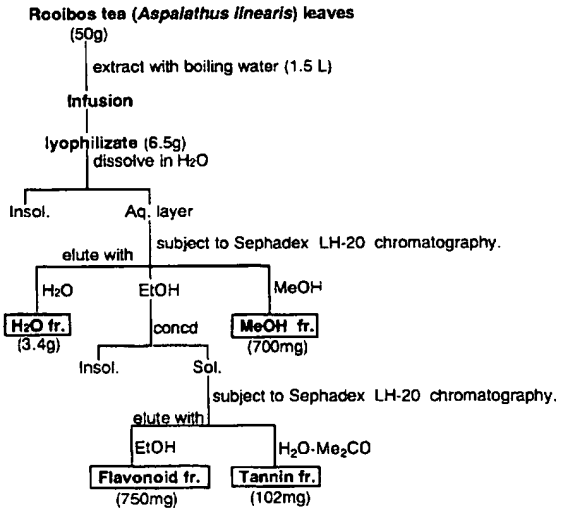

Fig. 1. Fractionation of rooibos tea infusion. The figures in parentheses show the yield of each fraction.

was prepared by extracting 50g of dried tea leaves with 1.5 l of boiling distilled water for 15 min. The tea infusion was lyophilized and the lyophilizate was then dissolved in a small amount of water and passed through a Sephadex LH-20 resin column. By elution with several solvents, the fractions of water, methanol, flavonoid and tannin were obtained. The flavonoid fraction was subjected to HPLC (Japan Spectroscopic) using a reversed-phase YMC-Pack D-ODS-5 column (20 mm (i.d.) × 250 mm, YMC) and an UV detector (JASCO 875-UV) at 280 mm. The elution solvent was a linear gradient ranging from H_2O to acetonitrile-H_2O (1:1) for 70 min and to 100% acetonitrile over 20 min at a flow rate of 5 ml/min. Eleven fractions (fr. 1–fr. 11) were obtained and analyzed. The effective fraction (fr. 11) was separated into two subfractions (fr. 11A–11B) and fr. 11B was further separated into five subfractions (fr. a–fr. e) by the same HPLC process (Fig. 4).

2.2. The micronucleus assay with mouse peripheral blood reticulocytes

This assay was performed according to the acridine orange-coated slide method developed by Hayashi et al. (1990). Eleven week-old male ICR mice (Japan SLC Inc. Hamamatsu, Japan) were used.

They were housed in an air-conditioned room and were given CE-2 commercial food pellets (Crea Japan, Tokyo) ad libitum. Five mice were assigned to each experimental group. One mg of each fraction suspended in 0.5% carboxylmethyl cellulose sodium was administered by gastric intubation 6hr prior to whole body γ-ray irradiation (1.5 Gy). The mice, anesthetized by pentobarbital (Nacalai Tesque, Kyoto, Japan; i.p.50 mg/kg), were exposed to ^{137}Cs rays at a dose-rate of 30R/min by PS-600SB irradiator (Pony Atomic Industry, Osaka, Japan). Five ml of peripheral blood was sampled from a tail blood vessel 42hr after irradiation. One thousand peripheral reticulocytes (type I,II) per mouse were scored and the number of micronucleated peripheral reticulocytes (MNRETs) was statistically analyzed by one-way ANOVA and Duncan's multiple range test.

2.3. Antioxidative assay

The antioxidative activity of each fraction and the HPLC-separated fractions was examined by the thiobarbituric acid (TBA) assay according to the method of Osawa et al. (1992). In this assay, Fenton's reagent (H_2O_2/Fe^{2+}) was used as an inducer of lipid peroxidation. Hydrogen peroxide and divalent iron generate hydroxyl radicals by the well known Harber-Weiss reaction. Briefly, methyl linoleate (10 μl) suspended in a buffer solution (0.2% SDS/0.05 M Tris-HCl/0.15 M KCl) was oxidized by Fenton's reagent-ferrous chloride (0.2 μmol/ml) and hydrogen peroxide (0.1 μmol/ml)-with and without sample. After the incubation of the mixture at 37°C for 16 h, 4% butylated hydroxytoluene (BHT) ethanol solution was added to prevent further oxidation. TBA was added to the reaction mixture and then the pigment produced was extracted with n-butanol. The absorbance of the butanol layer was measured at 532 nm with a spectrophotometer (Hitachi, U-3210).

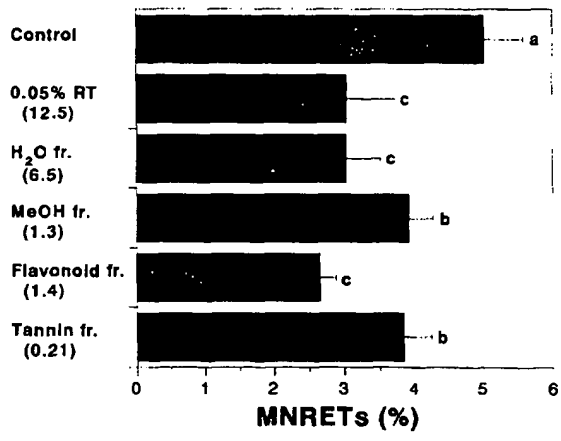

Fig. 2. Inhibitory effect of rooibos tea fractions on the induction frequency of MNRETs in γ-ray irradiated mice. Mice received a single gastric intubation of rooibos tea fractions 6hr before γ-ray irradiation (1.5 Gy). The dose of each fraction (mg/kg BW) was in proportion to the composition ratio of each fraction in the tea infusion. Values (mean ± SD) with different superscripts are significantly different ($p < 0.05$, $n = 5$).

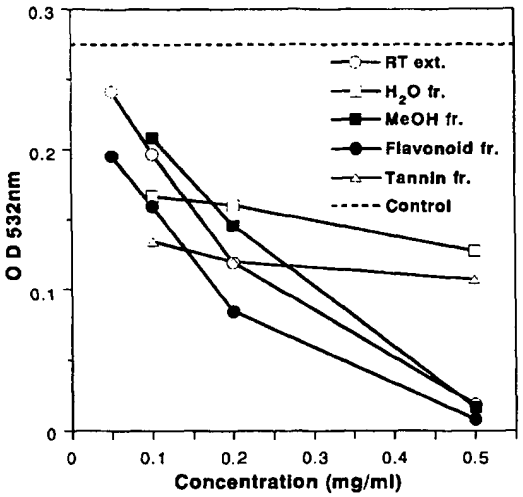

Fig. 3. Antioxidative activity of rooibos tea fractions. Each fraction was dissolved in distilled water and was subjected to the TBA assay.

2.4. Results

The effect of each fraction on micronucleus induction in γ-ray irradiated mice is shown in Fig. 2. The dose of each fraction was in proportion to the composition ratio of each fraction in the tea infusion. The flavonoid fraction showed the highest anticlastogenic activity and inhibited lipid peroxidation in a dose-dependent manner (Fig. 3). Fig. 4 shows the HPLC profile of the flavonoid fraction and the relative antioxidative activity (R.A.A.; 0.2 mg/ml). The small b fraction was most effective. From the b and c fractions, luteolin and quercetin were identified by

NMR spectral data, respectively. The chemical structure of these flavonoids is shown in Fig. 5.

3. Antioxidative and anticlastogenic activities of flavonoids

Luteolin, which is one of the flavonoids, was isolated from rooibos tea as an antioxidative and anticlastogenic component. In order to determine the relationship between both activities of flavonoids, 12 flavonoids were applied to the assays (See sections 2.2 and 2.3).

The flavonoids possessing higher antioxidative activity showed stronger anticlastogenic activity (Fig. 6). Luteolin was most effective against the inhibition of MNRETs induction by γ-ray and lipid peroxidation by Fenton's reagent. Next most effective was kaempferol. Quercetin tetra-methylether, which has methoxy groups instead of hydroxy groups at the

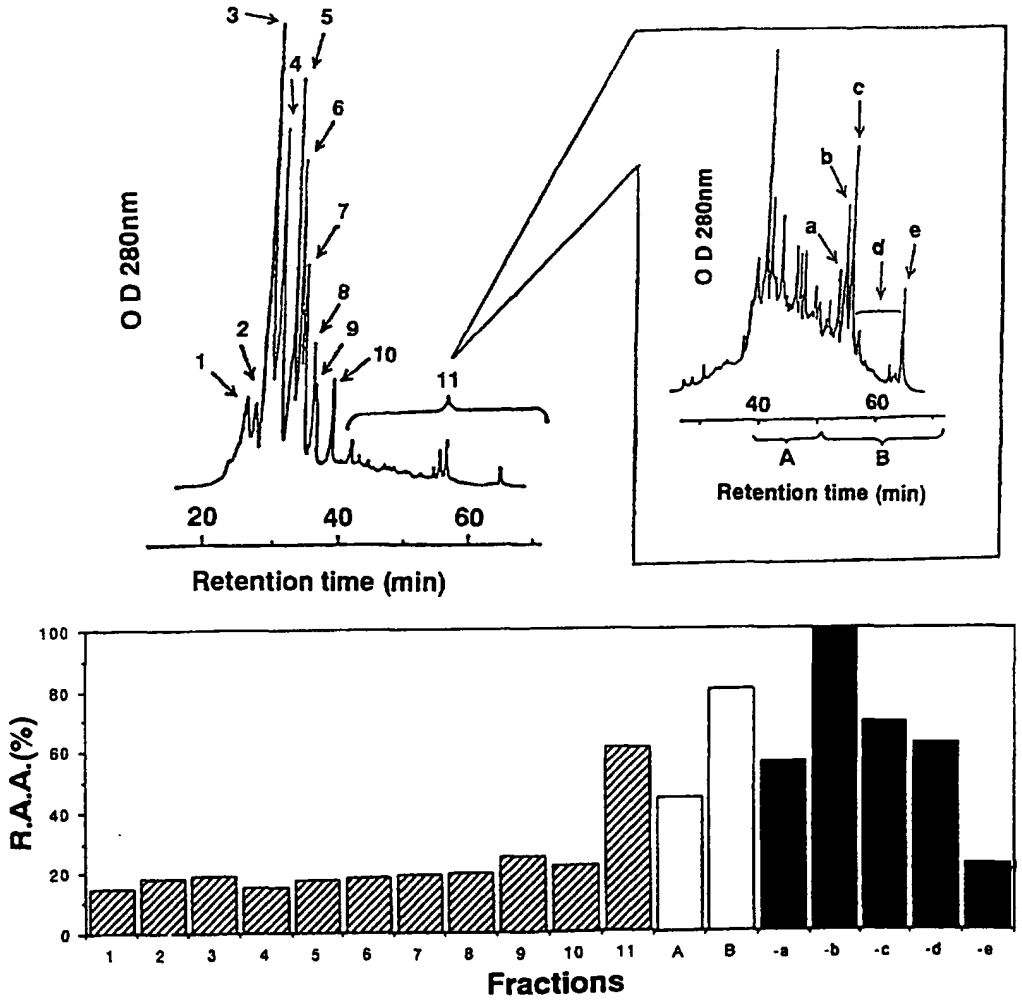

Fig. 4. HPLC elution profiles of the flavonoid fraction of rooibos tea and the relative antioxidative activity of component fractions. The flavonoid fraction was further purified by HPLC (HPLC conditions: column, YMC-Pack D-ODS-5; eluent, a linear gradient of acetonitrile/H_2O; flow rate, 8 ml/min; detection, UV 280 nm), and the relative antioxidative activity of the component fractions was assayed by the TBA method.

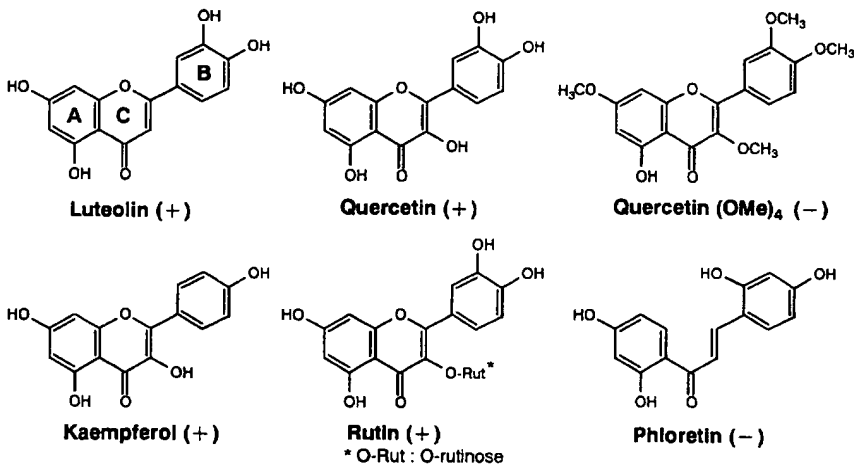

Fig. 5. Chemical structure of flavonoids.

3,7,3′,4′-positions, and phloretin, with an open C-ring, showed the least anticlastogenic and antioxidative activity. Their chemical structures are shown in Fig. 5. It is supposed that hydroxy groups of A and B rings and the closed C-ring of the flavonoid molecules play an important role in exhibiting these activities. Analysis of the data by a simple linear regression shows comparatively a good correlation ($r = 0.717$, $p < 0.01$) between both activities (Shimoi et al., 1994b).

4. In vivo antioxidative activity of luteolin

Numerous in vitro studies of flavonoids have demonstrated that flavonoids are strong antioxidative

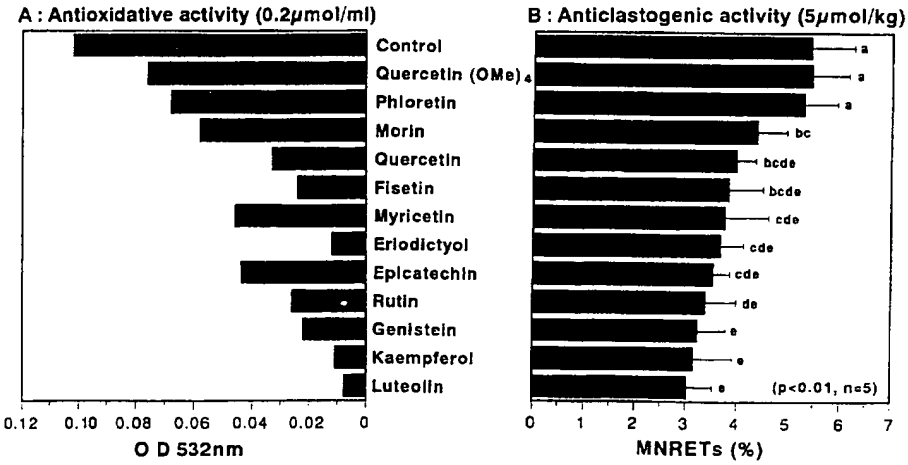

Fig. 6. Antioxidative and anticlastogenic activities of twelve flavonoids. (A) Values are means in duplicate (TBA method). (B) Mice received a single gastric intubation of flavonoids 6hr before γ-ray irradiation (1.5 Gy). Values (mean ± SD) with different superscripts are significantly different (micronucleus test).

agents. Their antioxidative activity is due to their ability to scavenge free radicals and to chelate metal ions (Robak and Gryglewski, 1988; Husain et al., 1987; Boyer et al., 1988). However, in vivo studies are few. Therefore, in order to discover the suppression potency of luteolin on the lipid peroxidation in bone marrow and spleen of γ-ray irradiated mice, a highly sensitive and simple chemiluminescence assay (Miyazawa et al., 1987) was carried out. Briefly, ICR male mice (11 weeks old) received luteolin (10 μmol/kg) 2 h before γ-ray irradiation. The bone marrow and spleen were removed 24 or 48 h after γ-ray irradiation (6 Gy) and homogenized in 0.04 M phosphate buffer containing 0.002% butylated hydroxytoluene. Fifty μl of luminol (0.002%) and cytochrome C (0.001%) were added to the 20% homogenized mixture (50 μl). The stainless steel well loaded with the mixture was placed just below the photocathode of the chemiluminescence analyzer (OX-7 TLA-66, Tohoku Denshi Sangyo, Japan) and the chemiluminescence intensity was automatically measured at room temperature. The content of protein was determined by the bicinchoninic acid method (Shihabi and Dyer, 1988). One-way ANOVA and Duncan's multiple range test were used for the statistics.

As shown in Fig. 7, the chemiluminescence intensity in both organs of the control mice increased significantly with time after γ-ray irradiation. However, when mice received luteolin by a single gastric intubation, significant suppression of the chemiluminescence intensity was observed in both organs ($p < 0.05$). The content of endogenous ascorbic acid in bone marrow after γ-ray irradiation (3 Gy) was also measured by the HPLC-ECD method (Umegaki et al., 1994a), because ascorbic acid is one of the endogenous antioxidants. Preliminary experiments show that the ascorbic acid content in bone marrow decreased significantly with time after irradiation. However, pretreatment with luteolin (10 μmol/kg) produced a mild decrease of ascorbic acid content in mouse bone marrow compared to that of the control. The protective effect of luteolin towards ascorbic acid was not significant, but there was a trend (data not shown). Ramarathnam et al. (1989a) have reported that rice hull prevented the loss of α-tocopherol in seeds irradiated with γ-rays. They isolated isovitexin, a C-glycosyl flavonoid, as an antioxidative component from rice hull (Ramarathnam et al., 1989b). Therefore, the protective effect of luteolin towards ascoprbic acid may be similar to that of isovitexin towards α-tocopherol. These results suggest that flavonoids work as antioxidants in vivo.

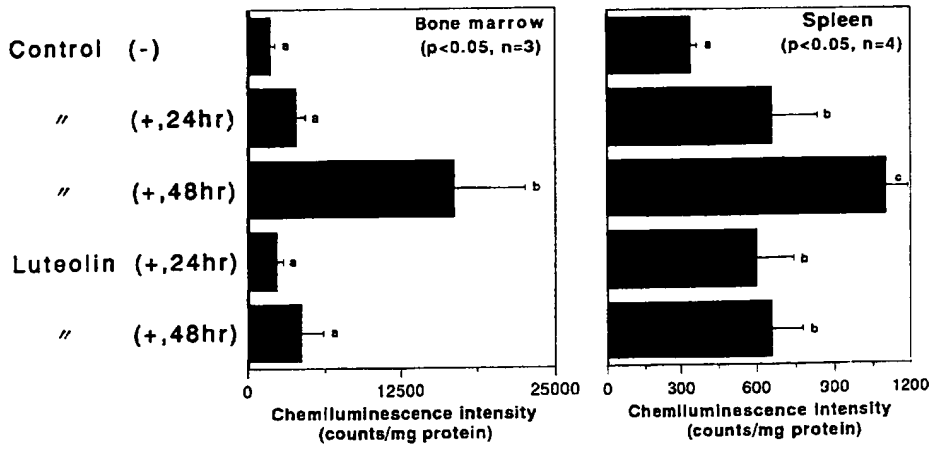

Fig. 7. Effect of luteolin on lipid peroxidation in bone marrow and spleen of γ-ray irradiated mice. Mice received a single gastric intubation of luteolin (10 μmol/kg) 2 h before γ-ray irradiation (6 Gy). Next, 24 and 48 h after γ-ray irradiation, the bone marrow and spleen were removed and homogenized. Luminol and cytochrome C were added to the mixture and the chemiluminescence intensity was automatically measured. Values (mean ± SD) with different superscripts are significantly different. (−): unirradiated. (+): irradiated.

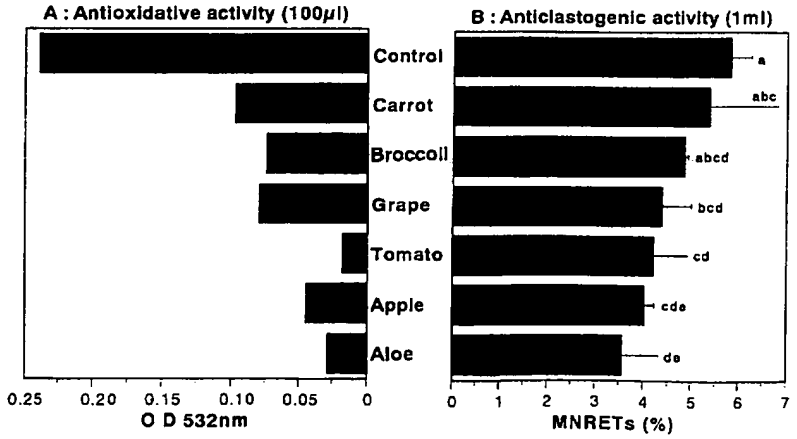

Fig. 8. Antioxidative and anticlastogenic activities of vegetable and fruit juices. The fresh juices were prepared using a mechanical juicer. Each juice (100 μl and 1 ml, respectively) was used for each assay. For other explanations see legend of Fig. 6.

5. Antioxidative and anticlastogenic activities of vegetable and fruit juices

Vegetables and fruits contain significant quantities of flavonoids. Their antioxidative and anticlastogenic activities were examined by the same method shown in Fig. 6. Vegetables and fruits were purchased from markets and washed with tap water. Only their edible parts were homogenized by a mechanical juicer (JC540A, Toshiba, Japan). The crude juice was centrifuged at 3000 rpm for 5 min and the supernatant was used for both assays.

The juices with more antioxidative activity showed more anticlastogenic activity (Fig. 8). Aloe (*A. arborescens Mill*) was most effective. It is clinicaly used for treatment of burns and peptic ulcers in Japan. The polyphenol extract from apples has demonstrated strong antioxidative activity in the autoxidation of linoleic acid (Tanabe, 1994). The flavonoids in vegetables and fruits may play a role, in part, in exhibiting both activities.

6. Conclusions

Numerous studies of flavonoids have demonstrated a wide variety of biological and chemical properties. Not only antioxidative properties but also antimicrobial (Nishino et al., 1987), anti-inflamatory-allergic (Middleton and Kandaswami, 1992), antimutagenic (Edenharder et al., 1993), anticlastogenic (Heo et al., 1992) and anticarcinogenic effects (Deschner et al., 1991; Verma et al., 1988; Fujiki et al., 1986) have been reported. Quercetin, a well known antioxidative flavonoid, has been demonstrated to be mutagenic and clastogenic in vitro (Sugimura et al., 1977; van der Hoeven et al., 1984) and to induce DNA strand breaks under certain reaction conditions (Yamada et al., 1985). However, lack of carcinogenicity (in rats) and clastogenicity (in mice) of quercetin has also been reported (Ito et al., 1989; MacGregor et al., 1987).

In this study, we isolated luteolin, which is one of the flavonoids, from rooibos tea infusion as a radioprotective component and showed its antioxidative activity in mice. A radioprotective effect of green tea catechins has also been reported (Hara, 1994). In a two year feeding study, a significant decrease in the incidence of lymphomas and an increase in survival were observed in γ-ray irradiated mice receiving a diet supplemented with EGCg, which is a strong antioxidant. Recently, chlorogenic acid, β-carotene and vitamins C and E have all shown a radioprotective effect against micronucleus induction in γ-ray irradiated mice (Abraham et al., 1993; Sarma and Kesavan, 1993) and human lymphocytes (Umegaki et al., 1994b). These antioxidants, including flavonoids, may play a role in scavenging free radicals, such as hydroxyl radicals induced with γ-rays, in mice. However, there is a possibility that pretreat-

ment with flavonoids induces tolerable function against oxidative stress. Further investigation is required to clear the action mechanism of plant flavonoids in vivo.

Kühnau (1976) estimated that the daily intake of mixed flavonoids per person is about 1 g. The study of the metabolic fate of [^{14}C] quercetin in rats has demonstrated that about 20% of the administered [^{14}C] quercetin was absorbed from the digestive tract and rapidly excreted into the bile and urine within 48 h (Ueno et al., 1983). The amount of the absorbed flavonoids was small, however, it is clear that the absorbed flavonoids exhibit radioprotective activity in vivo. These results may be related, in part, to epidemiological studies which have demonstrated the relationship between consumption of vegetables, fruits and tea with cancer prevention. In addition to the antioxidative vitamins, such as ascorbic acid and β-carotene, the flavonoids also seem to be important as antioxidants in the human diet and their consumption may be meaningful to our health.

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